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☐ 1: Appl Environ Microbiol 1986 Jul;52(1):142-5

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Chitinase determinants of *Vibrio vulnificus*: gene cloning and applications of a chitinase probe.

Wortman AT, Somerville CC, Colwell RR.

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To initiate study of the genetic control of chitinolytic activity in vibrios, the chitobiase gene was isolated by cloning chromosomal DNA prepared from *Vibrio vulnificus*. Chimeric plasmids were constructed from Sau3A I partial digests of chromosomal DNA by ligating 5 to 15-kilobase fragments into the BamHI site, i.e., in the Tcr gene, of pBR322 (Amr Tcr). The resulting plasmids were transformed into *Escherichia coli* DH1. Chitobiase activity of the insert-bearing clones was detected by using a chromogenic substrate, p-nitrophenyl-N-acetyl-beta, D-glucosaminide, and confirmed by the appearance of a fluorescent end product from the hydrolysis of 4-methylumbelliferyl-beta, D-N-N'-diacetylchitobiose. Endochitinase activity was demonstrated by liberation of water-soluble products produced by the degradation of [3H]chitin. Transformation of *E. coli* Y10R (lacY) with plasmids from chitinase-positive clones restored the lactose-positive phenotype, suggesting the presence of a permease associated with chitinase activity. Physical mapping of plasmids containing the chitinase determinants indicate that transcription of these genes in *E. coli* may be initiated at a *V. vulnificus* promoter.

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